

IN THE CLAIMS:

1. (currently amended) A method for characterizing proteins comprising:

a) providing:

i) ~~a sample comprising a plurality of proteins;~~

[[i]]i) a first separating apparatus that separates proteins based on a first physical property;

[[i]]ii) a second separating apparatus that separates proteins based on a second physical property;

[[iv]]iii) a mass spectrometry apparatus; and

iv) a sample comprising a plurality of proteins solubilized in a buffer, wherein said plurality of proteins are solubilized in said buffer and wherein said buffer is compatible with said first and said second separating apparatus, and wherein said buffer comprises a compound of the formula n-octyl C₆-C₁₂ glycopyranoside; and

b) treating said sample with said first separating apparatus to produce a first separated protein sample;

c) treating at least a portion of said first separated protein sample with said second separating apparatus to produce a second separated protein sample; and

d) directly feeding said second separated protein sample from said second separating apparatus to said mass spectrometry apparatus; and

e) mass spectrally analyzing at least a portion of said second separated protein sample with said mass spectrometry apparatus to characterize protein mass.

2. (original) The method of Claim 1, wherein said sample comprises a cell lysate.

3. (original) The method of Claim 1, wherein said first physical property is protein charge.

4. (original) The method of Claim 1, wherein said first separating apparatus comprises an isoelectric focusing apparatus.

5. (original) The method of Claim 1, wherein said first separating apparatus comprises a liquid phase separating apparatus.

6. (previously presented) The method of Claim 1, wherein said second separating apparatus comprises a reverse phase high performance liquid chromatography apparatus.

7. (previously presented) The method of Claim 6, wherein said reverse phase high performance liquid chromatography comprises non-porous reverse phase high performance liquid chromatography.

8. (previously presented) The method of Claim 1, wherein said mass spectrometry apparatus comprises an electrospray ionization-orthogonal acceleration-time-of-flight mass spectrometry apparatus.

9. (original) The method of Claim 1, further comprising the step of d) displaying at least said first physical property of at least a portion of said second separated protein sample.

10. (original) The method of Claim 9, wherein said displaying comprises a schematic representation of first and second physical properties of at least a portion of said second separated protein sample.

11. (original) The method of Claim 10, wherein said first and second properties comprise pI and hydrophobicity.

12. (original) The method of Claim 10, wherein said schematic representation comprises a 2-dimensional protein map, wherein said first physical property is represented on a first axis and wherein said second physical property is represented on a second axis.

13. (original) The method of Claim 10, wherein said schematic representation further displays protein abundance of proteins represented in said schematic representation.

14. (original) The method of Claim 13, wherein proteins are represented as bands in said schematic representation.

15. (original) The method of Claim 14, wherein protein abundance correlates to intensity of said bands.

16. (original) The method of Claim 14, wherein said schematic representation has a resolution that allows the differentiation of a first band representing a first protein and a second band representing a phosphorylated version of said first protein.

17. (canceled)

18. (previously presented) The method of Claim 1, wherein said buffer is further compatible with said mass spectrometry apparatus.

19. (canceled)

20. (previously presented) The method of Claim 1, wherein said compound of the formula n-octyl C₆-C₁₂ glycopyranoside is selected from n-octyl β -D-glucopyranoside and n-octyl β -D-galactopyranoside.

21-32. (canceled)

33. (previously presented) A method for characterizing proteins comprising:

a) providing:

- i) a sample comprising a plurality of proteins, wherein said sample comprising a plurality of proteins further comprises a buffer, and wherein said buffer comprises a compound of the formula n-octyl C₆-C₁₂ glycopyranoside;
- ii) an isoelectric focusing apparatus;
- iii) a non-porous reverse phase high performance liquid chromatography apparatus; and
- iv) a mass spectrometry apparatus;

b) treating said sample with said isoelectric focusing apparatus to produce a first separated protein sample, wherein said first separated protein sample is collected from said first separating apparatus in a plurality of fractions, each of said fractions defined by a distinct pH range;

- c) treating at least a portion of said first separated protein sample from at least one of said fractions with said non-porous reverse phase high performance liquid chromatography apparatus to produce a second separated protein sample; and
- d) mass spectrally analyzing at least a portion of said second separated protein sample with said mass spectrometry apparatus to characterize protein mass.

34. (currently amended) The method of Claim 3233, wherein said sample comprises a cell lysate.

35. (previously presented) The method of Claim 33, wherein said sample comprises a cell lysate.

36-38. (canceled)

39. (previously presented) The method of Claim 33, wherein said mass spectrometry apparatus comprises an electrospray ionization-orthogonal acceleration-time-of-flight mass spectrometry apparatus.

40. (original) The method of Claim 39, wherein said displaying comprises a schematic representation of first and second physical properties of at least a portion of said second separated protein sample.

41. (original) The method of Claim 40, wherein said first and second properties comprise pI and hydrophobicity.

42. (original) The method of Claim 40, wherein said schematic representation comprises a 2-dimensional protein map, wherein said first physical property is represented on a first axis and wherein said second physical property is represented on a second axis.

43. (original) The method of Claim 40, wherein said schematic representation further displays protein abundance of proteins represented in said schematic representation.

44-47. (canceled)

48. (previously presented) The method of Claim 33, wherein said compound of the formula n-octyl C6-C12 glycopyranoside is selected from n-octyl β -D-glucopyranoside and n-octyl β -D-galactopyranoside.

49. (canceled)

50. (original) The system of Claim 48, wherein said first separating apparatus comprises a liquid phase separating apparatus.

51-57. (canceled)

58-72. (canceled)

73. (previously presented) An automated method for separating proteins comprising:

a) providing:

i) a sample comprising a plurality of proteins, wherein said sample comprising a plurality of proteins further comprises a buffer, and wherein said buffer comprises a compound of the formula n-octyl C6-C12 glycopyranoside;

ii) an isoelectric focusing apparatus that separates proteins based on pH;

iii) a second separating apparatus that separates proteins based on a second physical property;

iv) a mass spectroscopy apparatus; and

v) an automated sample handling device comprising a switchable, multi-channel valve;

b) treating said sample with said first separating apparatus to produce a first separated protein sample, wherein said first separated protein sample is collected from said first separating apparatus in a plurality of fractions, each of said fractions defined by a distinct range of said first physical property;

- c) transferring said first separated protein sample to said second separating apparatus using said automated sample handling device;
- d) treating said first separated protein sample with said second separating apparatus to produce a second separated protein sample;
- e) transferring said second separated protein sample to said mass spectroscopy apparatus using said automated sample handling device; and
- f) mass spectrally analyzing said second separated protein sample with said mass spectroscopy apparatus to characterize protein mass.

74. (previously presented) The method of Claim 73, further comprising a centralized control network operably linked to said automated sample handling device, said first separating apparatus, said second separating apparatus, and said mass spectroscopy apparatus, wherein said centralized control network is configured to control said automated sample handling device, said first separating apparatus, said second separating apparatus, and said mass spectroscopy apparatus.

75. (previously presented) The method of Claim 73, further comprising providing a solid phase extraction apparatus, wherein prior to treating said first separated sample with said second apparatus; said first separated sample is treated with said solid phase extraction apparatus.

76. (previously presented) The method of Claim 73, wherein said sample comprises a cell lysate.

77. (previously presented) The method of Claim 73, wherein said first physical property is protein charge.

78-79. (canceled)

80. (previously presented) The method of Claim 73, wherein said second separating apparatus comprises a reverse phase high performance liquid chromatography apparatus.

81. (previously presented) The method of Claim 80, wherein said reverse phase HPLC comprises non-porous reverse phase high performance liquid chromatography.

82. (previously presented) The method of Claim 73, wherein said mass spectrometry apparatus comprises an electrospray ionization-orthogonal acceleration-time-of-flight mass spectrometry apparatus.

83. (original) The method of Claim 82, wherein said displaying comprises a schematic representation of first and second physical properties of at least a portion of said second separated protein sample.

84. (original) The method of Claim 83, wherein said first and second properties comprise pI and hydrophobicity.

85. (original) The method of Claim 83, wherein said schematic representation comprises a 2-dimensional protein map, wherein said first physical property is represented on a first axis and wherein said second physical property is represented on a second axis.

86. (original) The method of Claim 83, wherein said schematic representation further displays protein abundance of proteins represented in said schematic representation.

87-90. (canceled)

91. (previously presented) The method of Claim 73, wherein said compound of the formula n-octyl C6-C12 glycopyranoside is selected from n-octyl β -D-glucopyranoside and n-octyl β -D-galactopyranoside.